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Inheritance and Chromosome Number in the Gladiolus

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The garden hybrid gladiolus has probably a greater capacity for variation in its inheritance than any other cultivated plant. This would be expected from its ancestry, for it is derived from at least ten, probably more, entirely distinct species. Both cytological studies and breeding experiments give ample evidence of capacity for wide variability.

Observations of groups of seedlings of known and controlled parentage show that the following characteristics can be independently inherited: hooded or open bloom; with or without yellow, or red, violet or white color respectively in any combination; the color clear, or veined; or dark-flaked; throat yellow; blotched; lined; or dotted in any combination; with or without a pale zone around the throat; segments ruffled or plain; pointed or rounded tips; flower spikes stout or slender; with opposite or secund arrangement; close or wide spacing of blooms; few or many open at once; branched or simple; leaves bright or grayish green; and so forth through many other traits.

All of these varied traits can be directly traced to the different parent species: red-and-yellow flaked *G. psittacinus* with yellow throat; opposite-flowered white *G. oppositiflorus* with dark lined throat; purple-throated yellow *G. purpureo-auratus;* violet-tinted, dark-throated *G. papilio;* wide-open, white, dark lined *G. floribundus,* often somewhat ruffled; reflexed, wide-flowered scarlet *G. Saundersii* with red-spotted white throat; wide-open, rounded *G. cruentus,* blood red with dark blotch and white outer surrounding zone to the throat; *G. dracocephalus,* small hooded purple-spotted greenish yellow; deeply hooded yellow drooping *G. primulinus,* with its slender growth and long-stalked, soft-shelled cormels; and yellow-throated red *G. quartinianus,* very tall and late-flowering. Some authorities claim still other species as parents of the garden gladiolus, such as *G. cardinalis, G. blandus, G. tristis,* etc. The characters of any one species seem to be somewhat linked in the hybrids, so that one can frequently predict the growth habits from an observation of the flower form and coloring, but few cases have been observed of close linkage, to form definite races.

According to the chromosome theory of inheritance, most if not all of the inherited traits of a plant are transmitted by the chromosomes—tiny, dark-staining bodies evident in the cell nucleus during cell division. Usually the number of these formed in each cell by any kind of plant is constant, and each one seems to carry its own burden of the total inheritance of the plant. Detailed studies of Drosophila flies, daturas, and other organisms go to prove the soundness of this theory. Accordingly, chromosome studies of the gladiolus were undertaken, with the hope of learning more about its mode of inheritance, and of possibly finding an explanation of certain sterilities observed in some interspecific crosses.

For this study, pollen mother cells from the anthers of flower buds were used. Each mother cell nucleus divides into four nuclei, each of which then develops into the nucleus of a pollen grain. In the gladiolus this nuclear division takes place when the flower stalk is just emerging from the leaves, and there are really two steps in the process; first a division into two, then into four nuclei. During this process, the number of chromosomes in each nucleus is reduced from the number in the usual vegetative cells, to half that number in each pollen grain. The pollen mother cells at this stage are free in the cavity of the anther sac, and are easily squeezed out on a microscopic slide, stained and studied. The chromosomes stain black with aceto-carmine, but are very small; and in the early part of the telophase, after they are drawn away from the equatorial plate, they are short and bent nearly double, so that they are rather hard to count accurately. Counts were made after the first, and after the second division, when the chromosomes were in two or in four groups, and in each case the resulting count was the same, indicating that reduction division took place at the first division within the pollen mother cell. The results of these counts are as shown in table 1, page 67.

"Priority" is a hybrid derived from *G. primulinus* crossed with a garden variety of hybrid gladiolus. "Halloween" is a seedling of a cross of *G. quartinianus* with a Primulinus hybrid, and the remaining two are botanical species. The chromosome numbers were uniformly the same in all of these forms belonging to widely dif-

TABLE 1
Haploid Chromosome Numbers in Pollen Mother Cells of Gladioli.

		Number
Variety "Priority"[1]	10 observations	14
Variety "Halloween"	4 observations	14
Gladiolus *quartinianus*	2 observations	14
Gladiolus *tristis*	2 observations	14

ferent sections of the genus, and fourteen is assumed to be the haploid number for gladiolus, despite the fact that the only previously reported count, for the variety "La Muerthe," was recorded as 30.[2]

As has already been stated, there are many heritable traits of gladiolus, which are transmitted independently of one another. These may be assumed to be transmitted by different chromosomes. Since there have been fully 14 or more such independent characters observed among garden gladiolus hybrids, which can be freely interbred, we are free to assume, for purposes of study of the possibilities of different inheritance, that these independent characters are distributed over all of the 14 chromosomes. By many geneticists, each chromosome is regarded as a parcel, carrying its particular load of inheritable units, which are normally carried together, so that they are usually linked together in inheritance. Since the gladiolus has 14 chromosomes in each pollen grain and 14 in the egg cell of each ovule, the fertilized egg will have 28 chromosomes, 14 from the pollen parent and 14 from the seed parent. These 28 will be carried through the plant during its growth, each cell having thus 14 pairs of them, one of each of which will enter into its pollen grains or ovules. Each chromosome is assumed to be carried along intact from parent to offspring, except in such cases as may arise when chromosomes get tangled up during cell division, and portions of one pair may be exchanged. This is assumed to take place when there is crossing over, and severing of characteristics which are normally linked together. There are also numbers of other complications that occur in inheritance.

[1] Previously reported by me in THE GLADIOLUS REVIEW **4**: 17. 1927.
[2] Vilmorin, R. De, and M. Simonet. Nombre des chromosomes dans les genres *Lobelia, Linum* et chez quelques autres espèces végétales. C. R. Soc. Biol. Paris **96**: 166-8. Cited by Gaiser, L. O. Chromosome numbers in angiosperms II. Bibliographica Genetica **6**: 462. 1930.

All of these tend to add to rather than to subtract from the variability in inheritance. The simplest situation may be assumed to be that in which each character is determined by a single chromosome. So let us study the possibilities in the gladiolus on that basis.

If we take two strains or two species of gladiolus such that each breeds true for one of a pair of alternative characters, such as ruffled and plain petalled, and cross them, then the first generation will have in its constitution the tendencies for both characters. If, further, this trait is determined by a single factor, or gene, carried in one pair of chromosomes, then the hybrid will have the gene for ruffled in one chromosome, for plain-petalled in its mate. But when this hybrid produces pollen grains and ovules, and reduction takes place, only one of these chromosomes can enter into the male nucleus of each pollen grain or the egg cell of the ovule, so that each such reproductive cell can carry either the gene for plain-petalled or the gene for ruffled, not both. And so, if this hybrid is self-fertilized (or two sister hybrid plants are crossed, for hybrid gladioli are frequently self-incompatible), the offspring of the second generation may have each two chromosomes each carrying ruffled, or one ruffled and one plain-petalled or two plain-petalled. So there are three possibilities: ruffled, half-ruffled and plain-petalled.

If we now assume that the above two strains differ further in two other independent traits, as hooded and open flowered, then considering both these traits, the possibilities in the second generation are: Ruffled hooded, ruffled open, ruffled half-hooded, half-ruffled hooded, half-ruffled open, half-ruffled half-hooded, plain hooded, plain open and plain half-hooded. Thus there are 9 possibilities for two independent characters, or 3^2. Similarly, for three independent pairs of characters, there are 27, or 3^3 possibilities.

Since there are 14 pairs of chromosomes in the gladiolus we may assume that our two strains may differ by as many as 14 independent pairs of characters.

Of course, there can be only a limited number of combinations possible with twenty-eight different chromosomes, in fourteen pairs. If we designate the first pair of chromosomes as a and A, the second pair as b and B, and so forth, then we get the following set of chromosome units in the first generation hybrids:

hybrid X	a	b	c	d	e	f	g	h	i	j	k	l	m	n
	A	B	C	D	E	F	G	H	I	J	K	L	M	N

Since either a or A can enter into any pollen grain or ovule, combined with b or B, c or C, and so forth to n or N, as already stated, the possible kinds of reproductive cells possible in this first generation hybrid X are $2^{14} = 16{,}384$. But when two such reproductive cells unite, in fertilization, then the twenty-eight resulting chromosomes entering into the seed, and so carried into the second generation, may consist of a and a (aa), a and A (aA), A and a (Aa), or A and A (AA). Since aA and Aa are made up of the same constituents, they will be the same in result, so there are really three possibilities for the first or a chromosomes: aa, aA and AA. Similarly for the second or b chromosomes there are three possibilities, bb, bB, and BB, and so on for all of the others. So the number of possibilities of different combinations of twenty-eight chromosomes made up from two each of the fourteen sets of two, is $(3)^{14}$, or 4,792,869.

This means there are 4,792,869 possible different seedlings that might arise in the second generation from a cross between two different gladiolus species, if the two species differ from each other in at least fourteen characters, at least one of which is carried by each of the fourteen chromosomes. Whether any two species differ from one another to this degree is not easily proven, but garden hybrids seem to, so the 4,792,869 different progeny from a single cross are entirely reasonable.

If we go one step further, and, instead of intercrossing the seedlings of one interspecific cross among themselves, we cross two interspecific hybrids, each from entirely distinct species, so that in all, four distinct species enter into the heritage of the second generation, then the situation may be depicted by representing the chromosomes of the first second interspecific hybrid by letters and by numerals thus:

hybrid X	a	b	c	d	e	f	g	h	i	j	k	l	m	n
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
hybrid Y	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV

In this case, the 16,384 possible reproductive cells (pollen grains or ovules) of hybrid X may be assumed to differ from each of the 16,384 possible reproductive cells in hybrid Y. This would make the case more complicated, for then in the cross between X and Y the possible combinations would be for the first chromosome aa, aA, AA, al, Al, aI, AI, ll, lI, II, or a total of ten, the same number of combinations for the second, and so forth, making the possible number of different kinds of seedlings resulting from a hybrid made up from four such species 10^{14} or 100,000,000,000,000. Whether we would ever find four gladioli as radically different as is here assumed, and still find it possible to intercross them is not sure. So this is a purely hypothetical case—but even so the calculated results seem rather astounding—and just show what surprising possibilities there are along these lines. This computation takes account only of the different seedlings that might arise with a normal segregation of chromosomes. If chromosomes split abnormally, causing "crossing over" of characters normally linked on one chromosome then the numbers of possible combinations might be indefinitely increased. Of course, in genetical studies of gladioli or other plants nobody has attempted thus far to follow through as many as fourteen independent heritable characters in the manner indicated; no one garden or force of scientific workers would be large enough to make it practicable. At most, breeders work with two, three, or four characters of their plants, and even so they must grow thousands of seedlings before they can establish the manner of inheritance of these few traits.

The possibilities of different kinds of gladioli among seedlings, though, are so numerous that the tens of thousands of different seedlings raised each year by breeders are only a small fraction of the variations that may be expected among the modern hybrid strains, descended, not from four different species, but from at least ten. Nor is that the end; there are fully one hundred more gladiolus species that certainly have not yet been used in breeding. There may not be as many as 100,000,000,000,000 possibilities from any one set of hybrids, involving four species, because the species may be more nearly alike than was assumed in this calculation. But with so many different species that will readily intercross, the possibilities in hybrids are far greater than have yet been attained.

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